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Immunoreactive Plasma Cholinesterase (EC 3.1.1.8)[1]) Substance Concentration, Compared with Cholinesterase Activity Concentration and Albumin: Inter- and Intra-Individual Variations in a Healthy Population Group

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Summary: Substance concentrations of plasma cholinesterase (EC 3.1.1.8) were measured in 94 healthy individuals without occupational exposure to known inhibitors (six samples from each individual). Immunoreactive cholinesterase substance concentrations showed an inter-individual variation corresponding to CV_{total} = 22% (mean: 5.01 mg/l, SD: 1.11 mg/l). Intra-individual variations of immunoreactive cholinesterase substance concentration were correlated (r = 0.36) to intra-individual variations of albumin. Estimated by a repeated-measures analysis of variance, the *observed* intra-individual variation of cholinesterase substance concentration corresponded to CV = 8.8% (SD: 0.44 mg/l), which together with a CV_{error} = 6% (within and between runs), implies a *biological* intra-individual variation of cholinesterase substance concentration corresponding to CV_{intra} = 6.4%. Specific catalytic activity (kU/mg immunoreactive cholinesterase) was influenced by the ChE-1 phenotype (phenotype U: 1.58 kU/mg, phenotype UA: 1.22 kU/mg), but not by body weight, height, age, and sex. *Observed* intra-individual variation of specific catalytic activity corresponded to 6.4% (SD: 0.10 kU/mg), which together with an estimated CV_{error} = 6.2% implies the *biological* intra-individual variations of specific catalytic cholinesterase activity to be insignificant. The insignificant CV_{intra} makes specific catalytic cholinesterase activity a rational quantity for evaluation of unexpected fluctuations of cholinesterase activity concentrations.

Introduction

Plasma cholinesterase (pseudocholinesterase, butyrylcholinesterase, acylcholine acylhydrolase, EC 3.1.1.8) occurs in blood plasma as a number of distinct isozymes, predominantly represented by a globular, tetrameric (M_r: 340 000) structure (1–6). Biosynthesis of the 574-amino-acid-subunit (7) is controlled by the ChE-1 locus (8). Recently (9), the atypical, dibucaine resistant cholinesterase isoform has been shown to be an Asp-70 → Gly mutation.

Inter- and intra-individual variation of plasma cholinesterase activity concentration has been studied in healthy individuals (5, 10–14) and in patients with chronic liver diseases (10). All studies demonstrate substantial inter-individual variations, which are statistically related to physiological factors such as body weight, height, and sex, but also influenced by a variety of other physiological and pathological conditions (for review, see l. c. (4)); inter-individual variations of cholinesterase activity concentration are uninfluenced by particular electrophoretic components (5). Four of the studies (10, 11, 13, 14) demonstrate intra-individual variations in healthy individuals corresponding to a *biological* CV_{intra} = 5–5.5%; intra-individual variations of plasma cholinesterase activity concentration are independent of age, body weight, height, sex, or ChE-1 phenotype.

[1]) Cholinesterase, pseudocholinesterase, acylcholine acylhydrolase (EC 3.1.1.8).

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In health surveillance programmes, a decrease of plasma cholinesterase activity concentration in individuals who are occupationally exposed to organophosphorus pesticides, may readily be interpreted an indication of organophosphorus poisoning (15). To decide if such individuals really have been subjects to uncontrolled exposure, one must determine whether the decreased catalytic cholinesterase activity concentration is caused by a reduced cholinesterase substance concentration (e. g. due to reduced synthesis), or by a partial inhibition of the catalytic activity (e. g. due to systemic insecticides). In such situations, and as an alternative to measuring catalytic activity concentrations before and after in vitro reactivation, comparisons of specific catalytic cholinesterase activities (U/mg cholinesterase) have proved a useful diagnostic tool (*B. N. La Du*, Univ. of Michigan, Ann Arbor, personal communication).

Earlier studies indicate a close relation between cholinesterase activity concentration and cholinesterase substance concentration (16). However, no systematic studies of normal inter- and intra-individual variations of cholinesterase substance concentration (mg/l) or specific cholinesterase activity (U/mg) have so far been published. The aim of the present study is to describe such inter- and intra-individual variations of immunoreactive plasma cholinesterase substance concentration and specific catalytic plasma cholinesterase activity in a healthy population group without occupational exposure to known cholinesterase inhibitors; further, the study analyses the relative influence of five factors (ChE-1 phenotype, sex, age, body weight, and height) on these variations; finally, the observed variations of cholinesterase substance concentration are compared with the accompanying variations of albumin concentration.

Materials and Methods

Samples

Consecutive values of immunoreactive plasma cholinesterase substance concentrations, plasma cholinesterase activity concentrations, and plasma albumin substance concentrations (six samples from each individual) were obtained from 94 (43 males, 51 females, aged 20–65 years) of 193 apparently healthy individuals earlier studied (14). Five of the 94 individuals were of ChE-1 phenotype = UA; the remaining 89 individuals were found to be of ChE-1 phenotype U.

Analytical methods

Immunoreactive cholinesterase substance concentrations were determined by an enzyme immunoassay (16). Calibration was standardized with a highly purified human cholinesterase, which was quantitated according to *Lowry et al.* (17). Day to day reproducibility (M+D Monitrol I-E and Monitrol II-E): CV = 5.7% ($\bar{x}$ = 4.57 mg/l, SD = 0.26 mg/l, n = 34, lot no. LTD 210), CV = 6.2% ($\bar{x}$ = 3.45 mg/l, SD = 0.21 mg/l, N = 44, lot not. PTD 109).

Determination of plasma cholinesterase activity concentration was performed at 37 °C using butyryl thiocholine as substrate. Day to day reproducibility (M+D Monitrol I-E and Monitrol II-E): CV = 1.34% ($\bar{x}$ = 4.38 kU/l, SD = 0.059 kU/l, N = 125, lot no. PTD 108), CV = 1.92% ($\bar{x}$ = 6.38 kU/l, SD = 0.123 kU/l, N = 44, lot no. LTD 109), and CV = 1.36% ($\bar{x}$ = 6.51 kU/l, SD = 0.089 kU/l, N = 81, lot no. LTD 210).

Albumin substance concentration was measured by a turbidimetric immunoassay using a polyclonal (rabbit) anti-human albumin (Q328, Dakopatts, Glostrup, Denmark). The calibration was standardized with a commercial protein standard (Human Serum Protein Calibrator, lot no. 048, Dakopatts, Glostrup, Denmark). Day to day reproducibility (Seronorm™ Human, batch no. 1200, Nycomed, Oslo, Norway): CV = 3.9% ($\bar{x}$ = 37.8 g/l, SD = 1.49 g/l, N = 86).

Statistics

Stepwise multiple regressions, analyses of variance, and repeated-measures analyses of variance were performed on an IBM PC/AT using the SPSS/PC+ software package (SPSS Inc., Chicago, IL).

Results

Cholinesterase activity concentration and cholinesterase substance concentration

Table 1 shows plasma cholinesterase substance concentrations (mg/l immunoreactive cholinesterase), cholinesterase activity concentrations (kU/l), and specific catalytic cholinesterase activities (kU/mg immunoreactive cholinesterase) in the 94 healthy individuals studies. The table shows a higher immunoreactive cholinesterase substance concentration in individuals of ChE-1 phenotype U than in individuals of ChE-1 phenotype UA, and a higher cholinesterase substance concentration in males than in females. Specific catalytic cholinesterase activity was uninfluenced by sex, but significantly lower ($p < 0.001$) in UA individuals than in individuals of ChE-1 phenotype U.

The possible influence of sex, age, body weight, height, and ChE-1 phenotype on the inter-individual variation of cholinesterase substance concentration was analysed from a stepwise multiple regression ($\hat{y} = B_0 + B_1X_1 + B_2X_2 + \ldots\ldots + B_nX_n$; male = 1, female = 2, phenotype U = 1, phenotype UA = 2). Like catalytic cholinesterase activity concentrations (14), immunoreactive cholinesterase substance concentrations are significantly influenced by body weight, height, sex, and ChE-1 phenotype (tab. 2). Of the five factors, only the ChE-1 phenotype (multiple $r^2 = 0.739$) influences the specific cholinesterase catalytic activity. Table 3 shows the relative influence by the four significant factors on the immunoreactive cholinesterase substance concentration as assessed from a two-way ANOVA. Of the 51 females, 7 used oral contraceptives; the 7 women taking contraceptive pills showed a significantly ($0.01 < p < 0.05$) lower

Tab. 1. Cholinesterase activity concentration, substance concentration, and specific catalytic activity in healthy individuals, each represented by an average of six determinations performed within an eight-month period. SD in brackets.

	N	Cholinesterase substance concentration mg/l	Cholinesterase activity concentration kU/l	Cholinesterase specific activity kU/mg
Total	94	5.01 (1.11)	7.82 (1.77)	1.57 (0.10)
ChE-type U	89	5.06 (1.11)	7.96 (1.67)	1.59 (0.05)
Males	40	5.50 (1.21)	8.68 (1.81)	1.59 (0.04)
Females	49	4.69 (0.87)	7.40 (1.31)	1.58 (0.05)
ChE-type UA	5	4.13 (0.91)	5.08 (1.39)	1.22 (0.09)
Males	3	4.35 (1.11)	5.28 (1.77)	1.20 (0.11)
Females	2	3.81 (0.71)	4.78 (1.06)	1.26 (0.05)

Tab. 2. Major factors influencing inter-individual variations of plasma cholinesterase substance concentration (average of six determinations in each individual). Stepwise multiple regression analysis (male = 1, female = 2; ChE-1 phenotype U = 1, UA = 2).

Step	Variable entered	Multiple r	Multiple r^2	r^2 change
1	Body weight	0.421	0.177***	
2	+ Height	0.473	0.224	0.047*
3	+ Sex (1, 2)	0.525	0.275	0.051*
4	+ ChE-type (1, 2)	0.568	0.325	0.050*
5	+ Age	0.572	0.327	0.002 N.S.

Estimated regression coefficients (model: $y = B_0 + B_1 \cdot \text{weight} + B_2 \cdot \text{height} + B_3 \cdot \text{sex (1, 2)} + B_4 \cdot \text{ChE-type (1, 2)}$):

B_1: 0.048 ± 0.012
B_2: −0.064 ± 0.018
B_3: −0.85 ± 0.31
B_4: −1.10 ± 0.43
B_0: 15.17 ± 3.29

***: $p < 0.001$ *: $0.01 < p < 0.05$ N.S.: not significant

Tab. 3. Analysis of variance. Relative influence of covariates, sex, and ChE-type on plasma cholinesterase substance concentration in 43 males and 51 females (ChE-1 phenotype U or UA). Each individual represented by an average of six determinations.

Source of variation	Sum of squares	DF	Mean square	F
Covariates	25.79 (22.4%)	2	12.90	14.68***
Body weight	23.80	1	23.80	27.09***
Height	5.38	1	5.38	6.12*
Main effects	11.55 (10.0%)	2	5.78	6.57**
Sex	6.48	1	6.48	7.37**
ChE-type	5.67	1	5.67	6.46*
Two-way interactions				
ChE-type by sex	0.39 (0.3%)	1	0.39	0.45
Explained	37.73 (32.8%)	5	7.55	8.59***
Residual	77.31 (67.2%)	88	0.88	
Total	115.04 (100%)	93	1.24	

*: $0.01 < p < 0.05$ **: $0.001 < p < 0.01$ ***: $p < 0.001$

immunoreactive cholinesterase substance concentration ($\bar{x}$ = 3.83 mg/l, SD = 0.47 mg/l) than the 44 women who did not ($\bar{x}$ = 4.79 mg/l, SD = 0.86 mg/l).

Intra-individual variations of immunoreactive cholinesterase substance concentration were calculated from the six consecutive determinations in each individual. Like intra-individual variations of cholinesterase activity concentrations, the distribution of maximum intra-individual variations of cholinesterase substance concentrations (differences between highest and lowest substance concentration as a percentage of the mean concentration) was skew to the right (mean: 21%; range 6%−43%); in 15 of the 94 healthy vol-

Tab. 4. Repeated measures analysis of variance; tests involving observed within-subject effects. 94 individuals, six determinations in each individual.

Source of variation	DF	Cholinesterase substance			Cholinesterase activity		
		SS	MS	F	SS	MS	F
Sample (1 – 6)	5	0.73	0.15	0.74	0.74	0.15	0.77
Sex by sample	5	0.71	0.14	0.72	2.17	0.43	2.26*
ChE-type by sample	5	0.71	0.14	0.72	0.86	0.17	0.90
Sex by ChE-type by sample	5	1.44	0.29	1.46	1.68	0.34	1.74
Unexplained	450	88.81	0.20		86.55	0.19	

*: $0.01 < p < 0.05$

unteers the difference between highest and lowest immunoreactive cholinesterase substance concentration exceeded 30%. A quantitative estimate of the intra-individual variation was obtained from a repeated-measures analysis of variance. Table 4 shows an average intra-individual variation of immunoreactive cholinesterase substance concentration corresponding to SD_{intra} = 0.44 mg/l (CV_{intra} = 8.8%). Intra-individual variation of immunoreactive cholinesterase substance concentration was unrelated to sex or ChE-1 phenotype. The average intra-individual variation of catalytic cholinesterase activity concentration (CV_{intra} = 5.6%) was the same as previously reported (14).

Intra-individual variations of specific catalytic cholinesterase activity were assessed from immunoreactive cholinesterase substance concentrations and catalytic cholinesterase activity concentrations. Standard deviations calculated from the six determinations in each individual varied from 0.03 to 0.21 kU/mg. Repeated-measures analysis of variance showed an average intra-individual variation corresponding to SD_{intra} = 0.10 kU/mg (CV_{intra} = 6.4%), without influence by sex or by ChE-1 phenotype.

Albumin concentration

Plasma albumin concentrations were found to be higher ($p < 0.001$) in the 43 males ($\bar{x}$: 45.8 g/l; SD: 2.55 g/l) than in the 51 females ($\bar{x}$: 42.7 g/l; SD: 2.84 g/l); further, a stepwise multiple regression analysis showed an influence by age ($p < 0.001$) and by body weight ($0.01 < p < 0.05$). Women who used oral contraceptives exhibited a significantly ($p < 0.001$) lower albumin concentration ($\bar{x}$ = 40.2 g/l, SD = 1.96 g/l, N = 7) than women who did not ($\bar{x}$ = 43.1 g/l, SD = 2.78 g/l, N = 44). Albumin concentrations were uncorrelated to cholinesterase substance concentrations (r = 0.18).

Maximum intra-individual variations (differences between highest and lowest concentrations in percentage of a person's mean concentration) showed a distribution similar to that of cholinesterase; mean: 16.7%; range: 4.3% – 43%. Intra-individual variations of albumin concentration (expressed as CV) were correlated to intra-individual variations of cholinesterase substance concentration ($r = 0.36$, $p < 0.001$).

Discussion

In earlier studies, the immunoreactive plasma cholinesterase substance concentrations were reported to 5 – 15 mg/l (18 – 21). The present study shows a mean plasma immunoreactive cholinesterase substance concentration of only 5 mg/l (SD: 1.1 mg/l). Although there are various theoretical reasons for this discrepancy (for discussion, see l. c. (16)), the previously reported cholinesterase substance concentrations were probably overestimated. This suggestion is supported by comparison of the cholinesterase substance concentration (16) and protein concentration (17) in a highly purified cholinesterase preparation (made by Dr. *O. Lockridge*, Univ. of Michigan, Ann Arbor, using affinity chromatography on procainamide sepharose (22) combined with ion exchange), which showed that the immunoreactive cholinesterase substance concentration was 0.90 of that expected from the protein concentration.

Like cholinesterase activity concentration, immunoreactive cholinesterase substance concentration shows a substantial inter-individual variation. The same four factors that influence cholinesterase activity concentration (sex, body weight, height, and ChE-1 phenotype) influence immunoreactive cholinesterase substance concentration (tab. 3). Recently, body weight and height were also shown to influence alanine aminotransferase, aspartate aminotransferase, and γ-glutamyl transferase (23). The mechanisms giving rise to lower immunoreactive cholinesterase substance concentrations in individuals of ChE-1 phenotype UA than in matching individuals of ChE-1 phenotype U (tab. 2) are completely unknown (increased degradation?, decreased synthesis? decreased affinity of the monoclonal antibody to the UA isoform?). However,

although mechanisms involved are unknown, the described effects have to be considered, when comparing unmatched population groups.

Individuals exhibiting large intra-individual variations of plasma cholinesterase substance concentration also tend to exhibit large intra-individual variations of albumin concentration. Roughly estimated, one seventh (0.36^2) of the observed intra-individual variance of cholinesterase substance concentration may be explained from this relationship (parallel fluctuations of cholinesterase and albumin synthesis/degradation?, variations due to preanalytical errors?). Suggesting simple additivity of variances, an average *biological* CV_{intra} can be estimated from *observed* CV_{intra} and CV_{error}. Even though intra-individual variations differ from one individual to another, such estimates are crucial in diagnostic decision-making (24), and for defining appropriate analytical goals (25–26).

From the observed CV_{intra} (8.8%) and the actual analytical imprecision (CV_{error} = 6%), the *biological* CV_{intra} for cholinesterase substance concentrations may be estimated to be 6.4%. In a similar way, the corresponding biological CV_{intra} for cholinesterase activity concentration was calculated to be 5.4%; this estimate accords with other studies (10–11, 13–14) and with results obtained from studies of other plasma enzymes (26). From the actual CV_{error} for catalytic cholinesterase activity concentration and immunoreactive cholinesterase substance concentration, the actual CV_{error} of specific catalytic activity was calculated to be 6.2%. Combined with the *observed* CV_{intra} (6.4%), this implies an insignificant ($F = 1.07$, $p > 0.05$) *biological* CV_{intra} of the specific catalytic cholinesterase activity (1.6%). Intra-individual variations of cholinesterase activity concentration in healthy individuals are therefore due to varying cholinesterase substance concentrations, and not to varying inhibition of the catalytic cholinesterase activity. The insignificant CV_{intra} makes specific catalytic cholinesterase activity a rational quantity for evaluation of unexpected fluctuations of cholinesterase activity concentrations.

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